Phase transitions in phosphatidylcholine multibilayers

(^2H NMR spectroscopy)

PHILIP W. WESTERMAN*, MARIA JOÃO VAZ†, LAWRENCE M. STRENK†, AND J. WILLIAM DOANE†

*Molecular Pathology Program, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272; and †Department of Physics and Liquid Crystal Institute, Kent State University, Kent, Ohio 44242

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ABSTRACT The ^2H NMR spectrum of a multilamellar dispersion of 1-myristoyl-2-[14,14,14- $^2\text{H}_3$]myristoyl-*sn*-glycero-3-phosphocholine with 1 mol % cholesterol in excess water has been recorded at temperatures between -15°C and 36°C . Motionally averaged quadrupole coupling constants (ν_Q) and motionally induced asymmetry parameters η are obtained by spectral analysis. Values of these quantities indicate that, at temperatures below -4°C , any rotational motion of the molecules about their molecular long axis is slow on the NMR time scale. At temperatures immediately above the pretransition these same parameters show that a fast-rotational motion is occurring about the molecular long axis. This rotational motion is hindered in that the molecules flip about a twofold symmetry axis. Between -4°C and the pretransition, spectra appear as the superposition of two powder patterns, one corresponding to the pattern observed below -4°C and the other to the pattern above the pretransition. The relative contribution of the latter increases with temperature until the pretransition is reached. These data have been interpreted in two ways: either the sample between -4°C and the pretransition contains two populations of rapidly and slowly rotating molecules, or there is only a single population of molecules undergoing a 180° flipping motion on the time scale of the NMR measurement. The latter interpretation is more consistent with other experimental findings. At the temperature of the main transition the hydrocarbon chains melt. In the absence of cholesterol, spectra are more complex in that the line shape is reproduced by the superposition of three spectral powder patterns between -4°C and the pretransition and by the superposition of two spectral patterns above the pretransition. It is postulated that these two patterns observed above the pretransition are in direct correspondence to the two ripple structures observed by freeze-fracture electron microscopy in the absence of cholesterol.

The polymorphic phase behavior of synthetic phosphatidylcholines in excess water has been extensively studied by a wide variety of techniques (1–10). Although the thermodynamic parameters defining the subtransition,† pretransition, and main transition have been determined for a number of synthetic membrane lipids, no consistent picture of the molecular changes of the lipids during these transitions has yet emerged. Principally on the basis of x-ray diffraction techniques (12, 13), it has been concluded that, at temperatures below the pretransition, the hydrocarbon chains of fully hydrated phosphatidylcholines are fully extended, tilted with respect to the plane of the bilayer, and arranged with rotational disorder in a distorted quasi-hexagonal lattice. The pretransition is believed to be associated with the appearance of a two-dimensional monoclinic lattice consisting of lipid lamellae distorted by a periodic ripple (14–17). Several models for the ripple phase have been proposed (18–20). Saturation-transfer ESR experiments using nitroxide labels suggest that the pretransition marks the onset of

long-axis rotation (21), although NMR studies with perdeuterated chains suggest that this rotation also occurs below the pretransition (22). Finally, at the temperature of the main transition the hydrocarbon chains of the phospholipid are believed to melt and assume a liquid-like conformation (12). The details, however, of many features of this model suggested from the experiments above are not well defined (23–25).

A useful technique that has not been fully exploited in the study of phase transitions in model bilayer membranes is ^2H NMR spectroscopy. This technique has been applied in the study of membrane structure in the liquid-crystalline phase (26–28) but, apart from the publication of several spectra of multilamellar dispersions of lipids at temperatures below their main transitions (22, 29–31), no systematic study in the phase transition regions by using this technique has yet been reported. The present work describes a detailed ^2H NMR study of the phase behavior of multilamellar dispersions of 1-myristoyl-2-[14,14,14- $^2\text{H}_3$]myristoyl-*sn*-glycero-3-phosphocholine (deuterated dimyristoyl phosphatidylcholine, $\text{Myr}_2\text{-PtdCho-d}_3$) in excess water. The influence of 1 mol % cholesterol on the phase behavior of $\text{Myr}_2\text{-PtdCho-d}_3$ has also been studied. We have been able to detect by ^2H NMR the main, pre-, and subtransitions in these systems. In the pure phospholipid/water samples, the spectra appear as the superposition of two powder patterns in the P_β phase. This observation is consistent with freeze-fracture electron microscopic studies that show the presence of two ripple structures in pure $\text{Myr}_2\text{-PtdCho}$ samples. Also, in analogy with freeze-fracture studies in which one ripple structure disappears with the addition of ≈ 1.0 mol % cholesterol (17, 20), we find that only one spectral pattern survives when 1 mol % cholesterol is incorporated in the bilayer.

Our data for the cholesterol-containing sample confirm a model in which phospholipid molecules are rotating rapidly on the NMR time scale about their long axis in the P_β phase, and this motion is hindered in that molecules rotate by 180° flips. At the pretransition, upon cooling, our data indicate either an abrupt change in the rate of the hindered rotational motion so that it becomes on the time scale of the pulse echo sequence, or there is a rotational freeze-out for approximately half the molecules in the sample. At a temperature of -4°C , which we

Abbreviations: $\text{Myr}_2\text{-PtdCho}$, dimyristoyl-*sn*-glycero-3-phosphocholine; $\text{Myr}_2\text{-PtdCho-d}_3$, 1-myristoyl-2-[14,14,14- $^2\text{H}_3$]myristoyl-*sn*-glycero-3-phosphocholine. Consistent with the notation of ref. 12, the symbols L_α and P_β refer to the phases above the main transition temperature and between the pretransition and the main transition temperatures, respectively. Because we have observed an additional transition (subtransition), the phase existing below that transition is referred to as the L_β phase.

†The term "subtransition" used by Chen *et al.* (11) to describe the phase transition at 15°C in dipalmitoyl-*sn*-glycero-3-phosphocholine bilayers has been applied to the phase transition at -4°C observed by ^2H NMR in dimyristoyl-*sn*-glycero-3-phosphocholine (dimyristoyl phosphatidylcholine, $\text{Myr}_2\text{-PtdCho}$) bilayers. This phase transition in $\text{Myr}_2\text{-PtdCho}$ bilayers could not be detected calorimetrically (11).

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identify as the subtransition, our data show that rotation about the molecular long axis is slow on the NMR time scale.

MATERIALS AND METHODS

Synthesis of Deuterium-Labeled Phospholipid. 14,14,14-Trideuteriotetradecanoic (myristic) acid was prepared from methyl hydrogen tetradecanedioate (32). The purity of the fatty acid was confirmed by gas chromatography, the position of the ^2H label by ^{13}C NMR, and the extent of isotopic incorporation by mass spectrometry. Glycerophosphocholine cadmium chloride was prepared from egg lecithin according to Oldfield *et al.* (30). Glycerophosphocholine was acylated with tetradecanoic anhydride to yield $\text{Myr}_2\text{-PtdCho}$, using the procedure of Cubero Robles and van den Berg (33). Enzymatic removal of the fatty acid from position 2 (34) gave 1-myristoyl-*sn*-glycero-3-phosphocholine, which was acylated with 14,14,14-trideuteriotetradecanoic anhydride to yield $\text{Myr}_2\text{-PtdCho-d}_3$ (35). The product was isolated by column chromatography on silica gel, eluting with chloroform/methanol, 7:3 (vol/vol). $\text{Myr}_2\text{-PtdCho-d}_3$ was separated from the putative 1,3-dimyristoylglycerophosphocholine by collecting a large number of column fractions and discarding those that TLC showed were contaminated with the 1,3 isomer. The final product was rechromatographed on silica gel. TLC analyses were carried out on Adsorbosil-5 silica gel plates (Applied Sciences), using chloroform/methanol/water, 65:25:4 (vol/vol), as the solvent system, and both molybdenum blue (Applied Sciences) and iodine vapor to visualize spots.

Sample Preparation. A sufficient quantity of $\text{Myr}_2\text{-PtdCho-d}_3$ in chloroform was pipetted into a round-bottomed flask. In the case of the cholesterol-containing sample, a known volume of a standard cholesterol solution in the same solvent was also added. The chloroform was evaporated under reduced pressure and residual solvent was removed by the addition of dry ethyl ether followed by pumping under high vacuum for 24–48 hr. Deuterium-depleted H_2O was pipetted into the flask, which was first shaken on a Vortex stirrer and then, after the addition of several sterilized small glass balls, was gently agitated for 30 min in a water bath at 24°C . The resulting multilamellar suspension was pipetted into a 41.5×4.8 mm cellulose nitrate tube and centrifuged at $100,000 \times g$ in a Beckman SW 50.1 rotor, for 30 min at 24°C . Supernatant water was removed and the pellet was kept at -20°C for 24 hr, then at -80°C for a few days, and finally at -20°C for several hours, prior to recording the NMR spectra in the sealed centrifuge tube. After data acquisition, water content in the sample was determined gravimetrically and found to be 58–72% by weight. Lipid purity was checked by TLC. It is possible to detect the presence of a small amount of lysolecithin by overloading the TLC plate with sample. No results are reported on samples showing any detectable breakdown. Fatty acid analysis was carried out on a Hewlett-Packard 5840 A gas chromatograph using a column packed with 10% Silar-10C on Gas Chrom Q. The fatty acid content of $\text{Myr}_2\text{-PtdCho-d}_3$ released by alkaline digestion (36) was shown to be >99% tetradecanoic acid. Phosphate analyses of the phospholipid samples were performed by the method of McClare (37).

NMR Spectroscopy. Proton-coupled spectra were obtained at 30.87 MHz with a 10-cm bore high-resolution superconducting solenoid (Nalorac, Concord, CA) on a "home-built" four-phase spectrometer. A Nicolet 1080 data system (Nicolet Instrument, Madison, WI) and Biomation 805 digitizer were used to acquire and process the ^2H NMR spectra, using a 500- or 1,000-kHz effective spectral width and $2 \times 1,024$ data points. The $\pi/2$ pulse width was $4.5 \mu\text{sec}$, which is adequate power for the ^2H NMR spectra in all the systems investigated. Because the normal two-pulse quadrupole echo sequence (38) was af-

fected by acoustic ringing, a modified quadrupole echo sequence was used for data acquisition (39). Typically, the value of τ in the quadrupole echo sequence was $80 \mu\text{sec}$, and the pulse sequence was normally repeated three times per second. Spectra were recorded "on-resonance." An adequate signal-to-noise ratio was obtained from 1,000–5,000 transients in the L_α phase and from 15,000 transients in other phases.

Sample temperature was controlled to an accuracy of $<0.125^\circ\text{C}$ in a single-coil probe head with a temperature gradient $<0.15^\circ\text{C}$ across the sample, by a computer-controlled N_2 gas-flow system. After a 1°C change in temperature the sample was allowed 20–30 min to equilibrate. Each sample experienced the same thermal history, being first heated to $2\text{--}3^\circ\text{C}$ above the phase transition, then cooled to -15°C while spectra were recorded at $2\text{--}3^\circ\text{C}$ intervals, and finally heated from -15°C to 35°C while data were obtained at $0.5\text{--}2.0^\circ\text{C}$ intervals. The reported results are data acquired during increasing temperature runs. Spectra were finally recorded during a decreasing temperature run to establish the reversibility.

Spectral Simulations. For the L_α , P_β , and L_β phases of the cholesterol-containing sample, and the L_α and L_β phases of the pure $\text{Myr}_2\text{-PtdCho-d}_3$ sample, experimental spectral patterns could be fit to computer-generated spectral simulations made up of standard spherically distributed powder patterns (40). The fitting parameters associated with each powder pattern are the time-averaged coupling constant, $\langle\nu_Q\rangle$, and the motionally induced asymmetry parameter, η (41). Rounding of the edges and singularities of the patterns was accomplished by including a dipolar line-broadening contribution (41). The dipole-dipole contribution to line broadening was included in the standard manner by taking the line shape to be Gaussian with a frequency-dependent line width (42). The spectra recorded between the subtransition and the pretransition could not be fit to a single standard powder pattern as described above. These patterns could, however, be simulated in two different ways: (i) a superposition of two standard powder patterns, one of the type fitted in the P_β phase and another of the type fitted in the L_β phase. The relative intensities of each of these patterns were adjusted in the simulation. (ii) Powder patterns calculated for the intermediate-motion regime, in which the motional correlation times are comparable to the time scale of the deuterium quadrupole measurement. In this case, we applied a model in which the molecules are jumping 180° about a twofold axis (43). The expression for the spectrum, $I(\omega)$, given by Abragam (43), for the case in which ω_1 and ω_2 are the frequencies for the two different orientations of the $\text{C}-^2\text{H}$ bond and Ω is the exchange rate between the two orientations, has been modified to include a line-broadening term, ϵ . Thus,

$$I(\omega) = \frac{4\delta^2\Omega + 2\epsilon[(\omega - \bar{\omega})^2 + \delta^2 + 4\Omega^2] + 8\epsilon^2\Omega}{(\omega - \bar{\omega})^4 + 2(\omega - \bar{\omega})^2(2\Omega^2 - \delta^2) + \delta^4 + 2[(\omega - \bar{\omega})^2 + \delta^2](2\epsilon\Omega + \epsilon^2)},$$

in which $\bar{\omega} = (\omega_1 + \omega_2)/2$ and $\delta = \omega_1 - \omega_2/2$. These quantities are normalized by ν_Q whereby: the exchange rate $\Delta\Omega$ (Hz) = $\Omega\nu_Q$; the line width $\Delta\epsilon$ (Hz) = $\epsilon\nu_Q$; and the line splitting $\Delta\nu_i$ for each orientation, ($i = 1, 2$) at $\Omega = 0$ is given by $\Delta\nu_i$ (Hz) = $3\omega_i\nu_Q$.

From the above result, a spectral powder pattern of shape, $G(\omega)$ is calculated for a $\text{C}-^2\text{H}$ bond that makes an angle β with respect to the flip axis. $G(\omega)$ is given by

$$G(\omega) = 2 \int_0^{2\pi} \int_0^{\pi/2} I(\omega) \sin \Theta_0 d\Theta_0 d\phi_0.$$

In this case $\bar{\omega} = (3 \cos^2\beta - 1)(3/2 \cos^2\Theta_0 - 1/2) - 3/2 \sin^2\beta \sin^2\Theta_0 \cos 2\phi_0$ and $\delta = 3/2 \sin 2\beta \sin 2\Theta_0 \sin \phi_0$.

RESULTS AND DISCUSSION

It was found that ^2H NMR spectra recorded in the P_β phase were considerably simplified by the addition of 1.0 mol % cholesterol. For this reason we discuss these data first.

Fig. 1 shows four representative ^2H NMR spectra of a multilamellar suspension of $\text{Myr}_2\text{-PtdCho-d}_3$ and 1.0 mol % cholesterol in excess deuterium-depleted H_2O recorded at temperatures within the L_α , P_β , and L_β phases, as well as between the subtransition and pretransition. In the same figure, the various time-averaged quadrupole couplings (ν_Q) obtained from the spectra are plotted as a function of temperature.

In the L_α phase, a characteristic uniaxial ($\eta = 0$) powder pattern is observed with two 90° singularities separated by $\Delta\nu \approx 3 \text{ kHz} = \frac{3}{4}\langle\nu_Q\rangle$ [§] in basic agreement with the results of Oldfield *et al.* (28, 30). The value $\langle\nu_Q\rangle \approx 4 \text{ kHz}$ is considerably time averaged by the flexible wagging motion of the melted hydrocarbon chain (44).

In the solid, in which the chain and the molecule are rigidly ordered, and the *only* motional averaging is rotation of the C^2H_3 group about an axis colinear with the $\text{C}_{14}\text{—C}_{13}$ bond, the quadrupole coupling constant is estimated to be 51 kHz, corresponding to an observed splitting of 38.4 kHz. Such a value has been observed by Oldfield *et al.* (30) for $\text{Myr}_2\text{-PtdCho-d}_3$ bilayers at -170°C . This value agrees with that calculated from the quadrupole coupling constant of 170 kHz for the deuterium of each C^2H bond in the methyl group (45) and the angle that each C^2H bond makes with the $\text{C}_{14}\text{—C}_{13}$ threefold rotation axis.

In the L_β phase, the spectrum was fitted to a uniaxial powder pattern with $\langle\nu_Q\rangle \approx 40 \text{ kHz}$. The difference in this value and the "solid-state" value of 51 kHz could arise from residual *trans-gauche* isomerization of the hydrocarbon chain or from librational motions of the whole molecule. As can be seen from the data of Oldfield *et al.* (30), the singularities in the spectra observed in the L_β phase show greater broadening at -26°C than at -170°C , suggesting that the spectral lines in this phase are further broadened by mechanisms other than dipole-dipole interactions. One possible mechanism could be small-amplitude orientational librations about the molecular long axis that occur on the time scale of the NMR measurement.

In the P_β phase, a spectrum is observed that can be fit to a standard powder pattern, but it is one in which, unlike the L_α and L_β phases, the motionally induced asymmetry parameter (η) is nonzero. As illustrated in Fig. 1, the time-averaged coupling constant $\langle\nu_Q\rangle \approx 17 \text{ kHz}$. The measured temperature dependence of η is illustrated in Fig. 2 and shows $\eta \approx 0.25$ at 20°C . The nonzero value for η shows that the rotational motion in the P_β phase cannot be free rotational motion but must be hindered to some degree, involving 180° flips about the molecular long axis (41). It is instructive to compare the data in the P_β phase with that predicted by two ideal models: (i) a model in which the lipid molecule has the same rigidity as in the L_β phase and executes 180° flips about its long molecular axis; and (ii) a model in which the same rigid lipid molecule rotates freely about its long axis. In both models, ν_Q is the coupling constant associated with the molecule before the onset of rotation or flipping (i.e., $\nu_Q \approx 40 \text{ kHz}$). When the rate of rotation or flipping is large compared to ν_Q^{-1} , the coupling constant ν_Q becomes further time averaged to give a value $\langle\nu_Q\rangle$. In Fig. 3 are shown

FIG. 1. Temperature dependence of the time-averaged quadrupole coupling constant (ν_Q) and representative spectral patterns of $\text{Myr}_2\text{-PtdCho-d}_3$ with 1 mol % cholesterol in excess deuterium-depleted H_2O . The data were recorded at 30.9 MHz by Fourier transforming one-half of the quadrupole echo, obtained with a 0.3-sec recycle time, $2 \times 1,024$ data points, delay times $\tau_1 = \tau_2 = 80 \mu\text{sec}$, and a 90° pulse width $\approx 4.5 \mu\text{sec}$. Representative spectral patterns (a–d) for each phase are shown: spectrum a, -10°C , 1,000-kHz spectral width, 30,000 scans ($\bullet$); spectrum b, 13°C , 1,000-kHz spectral width, 30,000 scans; spectrum c, 19.5°C , 1,000-kHz spectral width, 15,000 scans ($\blacksquare$); spectrum d, 36°C , 500-kHz spectral width, 5,000 scans ($\triangle$). The small central peak in spectral patterns b and c was not present in all patterns in these temperature regions and appears to be an artifact. Error bars represent uncertainties in the fittings.

the values of $|\langle\nu_Q\rangle/\nu_Q|$ and η , calculated (41) for each case in which the molecule is executing 180° flips and rotating freely. These values depend on the angle β that the principal axis of the quadrupole interaction in the L_β phase makes with the rotation or flipping axis (molecular long axis). If the molecule were completely rigid, this angle would be that between the $\text{C}_{14}\text{—C}_{13}$ bond and the molecular long axis. Simple geometric considerations in such a case yield $\beta \approx 35^\circ$.

From Fig. 3 and the value $\nu_Q \approx 40 \text{ kHz}$, it is seen that both the free-rotation and flipping models yield $\langle\nu_Q\rangle \approx 20 \text{ kHz}$ for $\beta \approx 35^\circ$. For the same value of β , Fig. 3 shows values of $\eta = 0$ and 1 for the free-rotation and flipping models, respectively. To account for the observed values $\langle\nu_Q\rangle \approx 17 \text{ kHz}$ and $\eta = 0.25$ it is necessary to propose a model in which $\beta > 35^\circ$ and at the same time the flipping motion about the molecular long axis has some degree of free rotation—i.e., not a perfect 180° flip. An increased value of β could result from residual *trans-gauche* isomerizations of the hydrocarbon chain, while an increase in the degree of free rotation would result from small-amplitude librational motions imposed upon a fast 180° flipping motion.

Thus, our data, in the P_β phase, are consistent with a hindered rotational motion about the molecular long axis approximating to some extent a flipping type action. This interpretation is supported by data from aqueous dispersions of $\text{Myr}_2\text{-Ptd[methyl-}^2\text{H]Cho}$, which show no evidence for a finite value of η in the P_β phase. This result is expected from Fig. 3 if, as is widely believed (46), the principal axis of the time-averaged electric field gradient for rotating C^2H_3 groups in the head group of $\text{Myr}_2\text{-Ptd[methyl-}^2\text{H]Cho}$ is oriented at approximately right angles to the molecular long axis of rotation.

[§] In an aligned sample, the splitting is $\Delta\nu = \frac{3}{2}\langle\nu_Q\rangle|\frac{3}{2}\cos^2\theta_0 - \frac{1}{2}|$, in which θ_0 is the angle between the magnetic field direction and the principal axis of the time-averaged quadrupole interaction and $\langle\nu_Q\rangle$ is the time-averaged quadrupole coupling constant. In an unaligned sample the singularities of the powder spectrum occur at $\theta_0 = 90^\circ$ or $\frac{3}{4}\langle\nu_Q\rangle$. The quantity $\langle\nu_Q\rangle$ is related to the ν_Q , the solid-state quadrupole coupling constant, by $S\nu_Q = \langle\nu_Q\rangle$, in which S is the order parameter.

FIG. 2. (A) Temperature dependence of the relative intensities of the spectral patterns between the subtransition and the pretransition. (B) Temperature dependence of the values of the asymmetry parameter η for each of the powder patterns present in each phase. Symbols correspond to those of Fig. 1.

Our interpretation is also consistent with the experiments of Marsh (21) and the theoretical model of Scott (47) in which this phase is characterized by lipid molecules rotating about their molecular long axis. The possibility that motional averaging of the quadrupole interaction by translational diffusion can account for the observed value of $\langle\nu_Q\rangle$ is unlikely because of the slow translational self-diffusion rates of approximately 10^{-10} cm²/sec in the $P_{\beta'}$ phase (48).

FIG. 3. Calculated values (41) of $|\nu_Q/\nu_Q|$ and η for a 180° flipping motion (—) and free-rotational motion (---) on a time scale that is rapid compared to the ²H NMR measurement time ($\geq 1 \mu\text{sec}$). The angle β is the angle between the direction of the C—²H bond and the flipping or rotation axis.

Upon decreasing the sample temperature from the $P_{\beta'}$ phase, we observe in the vicinity of the pretransition the onset of another distinct spectral powder pattern, which accounts initially for approximately half of the total signal (Fig. 2) and has a value of $\langle\nu_Q\rangle \approx 40$ kHz, identical to that of the $L_{\beta'}$ phase. The contribution of this spectral pattern increases gradually with decreasing temperature (Fig. 2) until at -4°C there is another abrupt change, in which the spectral pattern assigned to the $P_{\beta'}$ phase disappears.

The spectra in the intermediate phase between the subtransition and the pretransition for the sample containing cholesterol is therefore characterized by two coexisting patterns, one corresponding to the pattern observed in the $P_{\beta'}$ phase and the other to the pattern found in the $L_{\beta'}$ phase. Experimental spectra have been reproduced in two ways. In the first, these two patterns were superposed, varying the relative contribution of each to the spectrum. Physically, this could mean that the sample contains two coexisting phases, one in which molecules are rotating slowly on the NMR time scale and the other in which rotation is fast. Such a model is in disagreement with the Gibbs phase rule for bilayer membranes in excess water (49).

A more likely interpretation of the line shape between the subtransition and the pretransition is a model in which the molecule undergoes a 180° flipping motion about its long axis at a rate $\Omega \approx \nu_Q^{-1}$. By extending the equations of Abragam (43), we have reproduced the observed spectra between the subtransition and the pretransition. Using a value of $\beta = 40^\circ$, with a frequency-dependent broadening contribution, experimental spectra have been reproduced by variation of the flipping rate (Ω). In this model, the phase between the pretransition and the subtransition therefore corresponds to the region where the rotational motion is in the intermediate-motion region on the NMR time scale. The subtransition is where the flipping motion changes from the slow to the intermediate region and the pretransition is the point where the rotation becomes fast on the NMR time scale. Although it is not possible with our data to make an experimental distinction, the second model is favored over the model of two coexisting phases for the following reasons: First, the model reproduces the observed line shapes in both the $L_{\beta'}$ and $P_{\beta'}$ phases. Second, the model is consistent with the Gibbs phase rule within and between the $L_{\beta'}$ and $P_{\beta'}$ phases, in that only a single phase is present. Third, the spectral line shape between the subtransition and the pretransition has been found to change slightly, on changing τ in the quadrupole-echo pulse sequence, from 80 to 500 μsec , a result consistent with the predictions of Spiess and Sillescu (50). Such a feature has been observed in ²H spectral powder patterns (51).

By fitting the experimental spectra with the theoretical spectra (refs. 43 and 50 and *Materials and Methods*), we have assigned flipping rates (Ω) over the temperature range -15°C to 20°C . An Arrhenius plot of $\ln \Omega$ versus temperature T^{-1} based on these data gives a straight line with slope 3.5 kcal (14.6 kJ)/mol between -4°C and 17°C , with distinct discontinuities occurring at these temperatures. These breaks in the plot suggest that the transitions are first order rather than second order. The subtransition has not been detected calorimetrically for Myr₂-PtdCho (11), probably because it occurs so close to the freezing point for water.

In Fig. 4 are shown four representative ²H NMR spectra of pure Myr₂-PtdCho-d₃ in excess deuterium-depleted H₂O, recorded at temperatures in the L_{α} , $P_{\beta'}$, and $L_{\beta'}$ phases as well as between the subtransition and the pretransition. Samples were prepared from the same synthetic lot of Myr₂-PtdCho-d₃ as that used for collecting the data in Fig. 1 except that no cholesterol was added.

The primary difference between these data and those of the

FIG. 4. Temperature dependence of the time-averaged quadrupole coupling consistent (ν_Q) and representative spectral patterns of pure Myr₂-PtdCho-d₃ in excess deuterium-depleted H₂O. The data were obtained under the same conditions as those of Fig. 1. The spectral patterns (a-d) were obtained at temperatures: a, -8.5°C (●); b, 13°C (■); c, 19.5°C (○); and d, 36°C (△).

sample containing 1 mol % cholesterol is the presence of three rather than two spectral patterns between the subtransition and the pretransition, with $\langle\nu_Q\rangle \approx 12$ kHz, 20 kHz, and 40 kHz. The L_α and L_β' phases are not noticeably affected, but there are now two coexisting spectral patterns in the P_β' phase. These patterns may correspond to the two different ripple structures observed by freeze-fracture electron microscopy for multilamellar samples of phosphatidylcholine that are quenched from temperatures within the P_β' phase (13-16). The two spectral patterns in the P_β' phase could arise from several models. One possibility is that there are two populations of rotating phospholipids, each in a distinct conformational state. If this is the case, the added 1 mol % cholesterol results in a rapid interconversion of the conformers, giving a $\langle\nu_Q\rangle$ that is the average of $\langle\nu_Q\rangle$ in the two conformers. Each ripple structure could correspond to a population of rotating lipid molecule in one of the two possible conformational states. It is not clear, however, at present, in which way the rotational state of the phospholipid molecules is related to the occurrence of the type of texture characterizing each ripple structure.

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